The Preoperative Neutrophil/Lymphocyte Ratio Does not Correlate with the 21-Gene Recurrence Score in Estrogen Receptor-Positive Breast Cancer Patients

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Introduction

Breast cancer (BC) is the most prevalent malignancy worldwide and the second leading cause of cancer-related death among women. Roughly 75% of these tumors are hormone receptor (HR) positive. In patients with estrogen receptor (ER)-positive, early-stage BC, the 21-gene recurrence score (RS) assay (OncotypeDX™; Genomic Health Inc., Redwood City, CA, USA) is widely employed to help determine appropriate candidates for adjuvant chemotherapy [1].

The RS ranges from 0 to 100 and constitutes a measure of the risk of distant relapse within 10 years for patients with ER-positive, node-negative BC, treated with adjuvant tamoxifen. It also predicts the individualized benefit of hormonal therapy and chemotherapy [1]. Currently, evidence suggests that the RS is prognostic for patients with ER-positive BC with node-positive disease as well [2]. This test is relevant in nearly half of all newly diagnosed BC patients. Therefore, its wide use can represent significant costs to healthcare systems, considering that most of the patients have early-stage and ER-positive BC.

The importance of systemic inflammation in promoting carcinogenesis and tumor progression is well recognized. Inflammation in the tumor microenvironment plays an important role in the proliferation and survival of malignant cells and was found to modulate tumor function, with both anti- and pro-tumor effects, in many solid tumors. Recent data suggest that systemic inflammation contributes to the development and progression of BC. Inflamed adipose tissue within the breast is associated with elevated levels of pro-inflammatory mediators, enhanced expression of aromatase (the rate-limiting enzyme for estrogen biosynthesis), and increased ER-α-dependent gene expression [3]. Numerous studies have established that elevated inflammatory markers, such as C-
reactive protein, the neutrophil/lymphocyte ratio (NLR), and the platelet/lymphocyte ratio, are associated with poor outcomes in cancer patients with different malignancies. Moreover, recent studies have demonstrated the negative impact of the elevated NLR on survival in BC patients. Considering that a complete blood count (CBC) is a routine pre-surgery examination, the NLR can be a simple and convenient measure of the inflammatory response [4].

The aim of this study was to examine the correlation between the pre-surgery NLR and the RS in patients with early-stage hormone-sensitive BC, for whom the NLR would be a faster and cheaper alternative to genetic testing, especially in countries with a low-funded healthcare system.

Materials and Methods

Patients

We retrospectively identified patients who underwent primary surgery for ER-positive BC at the Shaare Zedek Medical Center and who were referred for the RS assay between January 2006 and May 2012. We only selected patients who did not receive neoadjuvant chemotherapy. Demographic and clinicopathologic data were obtained from the medical records. Tumor size, lymph node status, HR status, human epidermal growth factor receptor 2 (HER2) status, and the CBC results were among the recorded data. The RS assay was performed on paraffin-embedded tumor samples at Genomic Health Laboratories, and found to be non-significant, as summarized in table 2.

The CBC was obtained after BC diagnosis and was confirmed prior to curative breast surgery or any anticancer treatment. Demographic and clinicopathologic data were obtained from the medical records. The RS assay was performed on paraffin-embedded tumor samples at Genomic Health Laboratories, as previously described [5].

The CBC was obtained after BC diagnosis and was confirmed prior to curative breast surgery or any anticancer treatment. The preoperative NLR was calculated as the quotient of the absolute neutrophil count divided by the absolute lymphocyte count (ALC). The cut-off value of 2.5 was decided upon according to receiver-operating characteristic (ROC) curves of previous studies [6]. The RS of each patient was categorized into a low-risk (≤ 2.0), an intermediate-risk (2.0–5.0), or a high-risk (≥ 5.0) group. The patients were placed into 3 groups based on the size of their tumors (≤ 2 cm, 2–5 cm, > 5 cm).

Results

Out of 312 reviewed cases, 242 had sufficient data for analysis and are included in this study.

The median age at diagnosis was 59.5 years, ranging from 27 to 84 years, and all but 2 patients were female. The tumors ranged in size from 0.50 to 5.50 cm, with a mean size of 1.8 cm; 73.2% of the tumors were < 2 cm in size. Most of the tumors (66.3%) were of grade 2; the rest was nearly equally divided between grades 1 and 3. Most (86.6%) were progesterone receptor (PR) positive and only 2 patients (0.8%) overexpressed the HER2. 22.3% had lymph node metastases (table 1).

The median RS was 18 (range 0–60). The mean NLR value was 2.11 (range 0.49–7.49). The NLR was < 2.5 in 71 (29.3%) patients. The NLR was not significantly correlated to the RS (Spearman’s p = 0.852).

We found a significant association between a high or low NLR and the tumor size (Fisher’s p = 0.042). Patients with larger tumors appeared to be less likely to have an NLR < 2.5 compared to patients with smaller tumors. ROC curve analysis did not yield a better single cut-off value for size. Other associations were examined and found to be non-significant, as summarized in table 2.

Subgroup analysis based on lymph node status, PR, tumor grade, and tumor size showed no significant correlation between the NLR and the RS (data not shown).

Discussion

Chronic inflammation is a key contributor to cancer development and progression. Cancer survivors with chronic inflammation may have an elevated risk of recurrence as a result of the effects of inflammatory processes on cell growth. In a multisite study of 734...
women treated successfully for early-stage BC, high levels of circulating acute-phase proteins, approximately 3 years after treatment, were associated with a 2-fold elevation in the risk of subsequent disease recurrence and mortality [7]. Azab et al. [8] pointed out that tumor-associated neutrophils initiate cascades that can lead to enhanced angiogenesis and tumor growth, while lymphocytes can induce apoptosis of cancer cells. In addition, tumor-infiltrating lymphocytes (TILs) have been associated with better prognosis.

Neoadjuvant therapy provides an opportunity to study the relationship of the immune system to chemotherapy response and survival. Denkert et al. [9] analyzed 1,056 pretreatment biopsies of patients in 2 prospective neoadjuvant trials, for TILs and stromal TILs (sTILs). A high percentage of tumor cell nests with TILs independently predicted for pathologic response. The study suggests that a high percentage of TILs can be useful to predict patients who will do very well with anthracycline/taxane-based neoadjuvant therapy, while those with low TIL percentage should be considered for other approaches including immunologic ones. A recent study examined the resected specimens of 278 patients who received neoadjuvant chemotherapy for triple-negative (ER-negative, PR-negative, and HER2-negative) BC (TNBC). A higher percentage of tumor cell nests with TILs or a higher percentage of peritumoral stromal area occupied by sTILs was associated with an absence of metastatic axillary lymph nodes and a small tumor size (< 2 cm). Furthermore, increased percentages of both TILs and sTILs were found to be positively correlated with metastasis-free and overall survival in the entire cohort, but not in the subset with node-negative and ≤ 2 cm residual disease. 19 out of 27 patients with high-TIL residual disease were evaluated for TILs on the diagnostic biopsy. With only 1 exception, the TIL percentage in post-chemotherapy surgical specimens was higher compared to that in pre-chemotherapy specimens, suggesting that the inflammatory process plays a part in the efficacy of antitumor drug therapy [10].

An analysis of 368 patients consisting of 2 cohorts of ER-negative patients treated with anthracyline-containing neoadjuvant chemotherapy for BC showed that, for the HER2-positive and triple-negative patients, a high level of TILs was significantly correlated with achieving pathologic complete response [11]. The results from these studies suggest that inflammation plays an important role in the prognosis of breast cancer, particularly in highly proliferative tumors such as triple-negative and HER2-positive subtypes.

In the adjuvant setting, TILs were evaluated in 2,009 node-positive patients in the Breast International Group (BIG) 02–98 trial of 4 taxane- and non-taxane-containing anthracyline regimens. With a median follow-up of 8 years, there was a significant correlation between increased TIL percentage and longer survival and lower relapse rate in the 256 patients with triple-negative disease [12]. In the accompanying editorial, Denkert [13] points out that the evaluation of TILs can be done routinely during standard histologic examination and that in fact TIL presence has been in use for years to diagnose medullary BC. This immune phenomenon, which is the subject of ongoing investigations, suggests that immunotherapy to augment TIL activity may be a useful approach to treatment in selected patients.

Recently, systematic reviews and meta-analyses demonstrated an association between a high NLR and worse long-term outcomes following curative-intent surgery [14]. There is no consensus optimal cut-off value of the NLR for reliably classifying patients as having high or low risk for recurrence and predicting survival. In a retrospective analysis, Noh et al. [6] found that an NLR ≥ 2.5 at initial presentation of luminal A subtype BC was an independent predicting factor for poor 5-year survival (87.7% vs. 96.7% for NLR < 2.5). Azab et al. [8] found a high pretreatment NLR to be significantly predictive of increased 5-year mortality in 437 women with primary BC, regardless of the ALC and independent of having received chemotherapy as part of treatment. In another retrospective analysis, this group found BC patients with NLR > 3.3 to be at significantly increased risk for 1-year (16% vs. 0%) and 5-year (44% vs. 13%) mortality, compared to those with an NLR < 1.8. The increment in risk remained after adjusting for older age and advanced stages of cancer [15]. In a study of 157 HR-positive HER2-negative BC patients receiving neoadjuvant chemotherapy with a mean post-surgery follow-up of 21 months, an NLR of over 2.25 correlated with poor relapse-free and overall survival and was shown to be an independent prognostic factor [16]. In contrast, Cihan et al. [17] analyzed 350 patients who underwent primary surgery and radiotherapy for BC and did not find a correlation between the NLR or other pretreatment inflammatory markers and disease-free or overall survival. The authors speculated that the reasons for lack of correlation may have been the relatively short follow-up period and the preponderance of early-stage BC.

Dirican et al. [18] retrospectively examined the performance of the preoperative NLR as a prognostic factor in BC in 1,527 patients from a single institution, with almost 6 years of follow-up. Using a cut-off value of 4, the NLR was found to be an independent predictor of disease-free survival and overall survival. The NLR was also significantly associated with the tumor stage.

Molecular pathways involved in fat metabolism and obesity appear to be instrumental in the development and progression of BC. 1 study including 167 Japanese patients with early-stage BC showed that the NLR was higher in patients with lower body mass index (BMI) [19]. Data concerning the BMI were not collected in our study. It is known that inflammatory markers are linked to the BMI.

In the current study, we report the absence of a statistically significant correlation between the Oncotype DX™ 21-gene RS and the NLR in 242 consecutive BC patients with ER-positive disease, treated in a single medical facility. The major limitation of our study is that it is a retrospective, single-center study. Although previously studied and currently available theories would support the hypothesis that such a correlation exists, there are several possible explanations of its absence in the current study.

The Oncotype DX™ gene array utilizes real-time polymerase chain reaction (RT-PCR) assays, a molecular amplification technique that is very sensitive but disregards tissue morphology. As a result, contamination of tumor mRNA with non-neoplastic tissue or biopsy cavity material may occur [20]. Previous studies have shown evidence supporting contamination, such as the presence of
increased stromal cellularity and/or associated inflammatory cells in low-grade invasive breast carcinoma specimens, which may contribute to an apparently increased risk of recurrence according to the RS [20, 21].

Previous studies exploring the NLR in BC measured clinical outcomes such as disease-free survival or overall survival and not a molecular prognostic profile [6, 8, 15]. This may suggest that the NLR predicts prognosis independently of the assessed molecular profile. Furthermore, the study sample of previous publications was more heterogeneous and included larger proportions of patients with advanced disease (e.g., metastatic disease and involvement of more than 3 axillary nodes) and HER2-positive and ER-negative tumors [6, 8, 15]. A recent study showed a significant role of inflammation in TNBC, specifically in cases with node-positive and/or larger residual disease, suggesting that the NLR is biologically more relevant in these patients [9].

It is possible that the NLR and RS represent different pathways of tumor biology; the OncotypeDX™ assay characterizes tumor cells while the NLR is a feature of their microenvironment. It therefore might be prudent of the clinician to use both, along with other clinical and laboratory features, to assess the risk of recurrence.

Conclusions

Although the current study failed to establish a relationship between the NLR and the RS, additional research is required to assess the usefulness of the NLR as a prognostic tool in BC. The NLR may potentially offer an inexpensive aid to gene assays in predicting recurrence. In the future, it will be of great interest to correlate the NLR and RS with disease outcome.

Disclosure Statement

The authors have no conflict of interests.

References